

ASSESSMENT OF THE PLANT EXTRACTS BIOLOGICAL ACTIVITY IN THE PROTECTION OF SEEDLINGS OF *CUCUMIS SATIVUS* L. FROM POWDERY MILDEW

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Key words: powdery mildew, *Rheum officinale* Baill. (*Polygonaceae*), *Azadirachta indica* Juss. (*Meleaceae*), plant extracts, *Cucumis sativus* L.

INTRODUCTION

In order to produce organic products, there was an obvious need to search for new, alternative, environmentally friendly methods of combating diseases and pests, which led to the recognition of plant extracts as which led to the recognition of plant extracts as highly effective plant protection products, socially acceptable, biodegradable and targeted to specific pathogens. We used rhubarb extracts (roots and leaves) and neem extract (seeds) in our experiments. The synthesis of phenolic compounds is a chemotaxonomic feature of the *Polygonaceae* family plants, in particular of the genus *Rheum*, the bioactive substances of which have been well studied, due to its active use in medicine and food industry for the last three thousand years in various countries [1]. So, already more than 100 different phenolic compounds have been identified or conditionally characterized in six rhubarb species - *R. officinale*, *R. palmatum*, *R. tanguticum*, *R. franzenbachii*, *R. hotaense* and *R. emodi*. Modern phytochemical studies of rhubarb in China, Korea, America, India have established the main biologically active ingredients of phenolic compounds, including anthraquinones (physcion, chrysophanol, emodin, aloe-emodin and rhein and their glucosides), anthocyanins (cyanidin 3-rutinoside and cyanidin 3-glucoside), flavanoids (catechin 3, quin -Orhamnoside, quercetin3-O-galactoside, and quercetin 3-O-rutinoside), stilbene (trans-rhapontigenin) and deoxyrapontigenin (cis-rhapontigenin, resveratrol and piceatannol) [2-3].

An ethanol extract from the roots of *R. officinale* Baill in a liquid formulation was used by Xiaojun Yang (2009) from China to control pathogen of powdery mildew (*Podosphaera xanthii*). The efficacy of the product was evaluated during tests under controlled conditions in growing vessels, as well as in open and protected ground for 2 years. In most studies, the effectiveness has reached over 80%. The cucumber yield during processing was 53,1% higher compared to the untreated control. These results showed that the *R. officinale* Baill plant

extract can be an effective alternative plant protective agent in the complex and biological control of cucumber powdery mildew [4]. These data are confirmed by the works of Keinath A. P. (2012), in the "Crop Protection" journal [5]. The effect of *Rheum palmatum* root extract in the control of leaf infection (*Plasmopara viticola*) in the susceptible variety *Vitis vinifera*, investigated by Godarda S. et al. (2009). They found that this natural product is toxic to the pathogen and causes a protective reaction in grapes in the form of phytoalexin accumulation, increased peroxidase activity, and a hypersensitive reaction. The inhibition of the first stage of development of biotrophic hyphae *Plasmopara viticola* by plant extract was observed. HPLC-DAD-MS analysis showed that this extract contains many phenolic compounds belonging to the anthraquinone family, for example, emodin, aloe-emodin, chrysophanol and physcion, which are recognized as active ingredients [6]. The mechanism of the *R. emodi* root extract action as an activator was investigated by a group from India, led by Mauryaa S. (2010). They proved the extract to be very effective in the preventive and curative treatment against powdery mildew (*Erysiphe cichoracearum*) of balsam (*Impatiens balsamiana*) in the field. Analysis of leaves treated with *R. emodi* root extract proved that the preventive effect of the extract was associated with induced resistance to powdery mildew based on an increase in the synthesis of phenolic acids in leaves treated with the extract [7].

Among the botanical insecticides currently marketed - *Azadirachta indica* Juss. (*Meleaceae*) is one of the least toxic to humans and has a very low toxicity to beneficial organisms, therefore it is very promising for combating many phytopathogens. The obnoxious odor of neem oil is ascribed to the presence of sulfur-containing volatile compounds [8]. The important bioactive compounds of neem seed oil belong to the limonoid class of triterpenoids, such as azadirachtin (azadirachtin A), salannin, salannol, nimbin, gedunin, 3- tigloylazadirachtol (azadirachtin B), epoxyazadiradione, 17 β - hydroxyazadiradione, 1-tigloyl-3-acetyl-11-hydroxymeliacarpin (azadirachtin

D), 1 α ,2 α -epoxy-17 β - hydroxyazadiradione, 1 α ,2 α -epoxynimolicinol, and 7- deacetylnimolicinol (Hallur et al., 2002; Ismadji et al., 2012) [31, 36]. In 1942, Siddiqui reported bitter principles, nimbin, nimbinin, and nimbidin, where nimbidin was the major bitter principle of neem seed oil [9-10]. The results of a study by Ismail OM (2016) showed the effectiveness of neem oil in the fight against mango powdery mildew (*Mangifera indica* L.) and elimination of malformations of culture [11]. The effective dose at which the growth of *Monilinia fructicola* is inhibited by 50% has been evaluated for neem oil in laboratory experiments by researchers Lalancette N, McFarland K.A. (2015) [12]. Based on the foregoing, the purpose of the work was outlined: To determine the possibility of using plant extracts from rhubarb (roots and leaves) and neem (seeds) plants as alternative means of protecting *Cucumis sativus* L seedlings from powdery mildew.

MATERIALS AND METHODS

The subjects of our research were bioactive substances extracted from plants *Rheum officinale* and *Azadirachta indica*. The study used the roots and leaves of hand-picked rhubarb plants. Stored and processed plant raw materials, dried to an air-dry state, in accordance with the requirements of regulatory enactments [13]. We used German-made Neemöl-Rimulgan oil. The bioactive component nimbin imparts fungicidal properties to the neem seed oil extract [14].

The object of research - powdery mildew appears in the form of white powdery spots, first on the upper and then on the lower side of true cucumber leaves. With severe damage, the leaves and stems are completely covered with a mealy bloom, the leaves turn yellow, turn brown, become brittle and dry out. On the stems, the plaque of sporulation is manifested only at the last stages of epiphytity. The most favorable conditions for the development of the disease are high humidity, about 60-80% and air temperature, within 18-20°C. Productivity due to disease can decrease by 40-50%.

Method for the identification of *Cucumis sativus* powdery mildew pathogens based on the conidial stage

Perithecia (fruiting bodies) are formed annually in large quantities on all major types of pumpkin crops and, having overwintered on plant residues, can cause infection. At the beginning of summer, ascospores, freed from their bags, are carried by the wind. Once on the host, they germinate and develop primary mycelium. Our studies to identify the pathogens of powdery mildew on *Cucumis sativus* were carried out in accordance with the methods described in the works of Sokolov [15]. Conidia were examined microscopically, photographed and used to create a suspension. Suspensions of conidia were used for artificial infection of plants in experiments.

Method for determining the biological effectiveness of plant extracts for the control of powdery mildew on *Cucumis sativus* seedlings

To assess the biological effectiveness of the treatment of *Cucumis sativus* seedlings with *Rheum officinale* plant extracts (roots and leaves) for the control of powdery mildew, artificial infection of seedlings in the phase of 2-3 true leaves was used. In each variant, 10 plants were studied in 4 replicates. Sources of infection were powdery mildew-infected leaves of cucumber from a greenhouse. The spores of the fungus were washed off with distilled water, and the solution was filtered. The concentration of the suspension was 100 thousand conidia in 1 ml of solution. The plants treated with extracts were infected with powdery mildew pathogen after 4 and 72 hours. The plants treated with the extracts were infected with a suspension of the fungus, whereupon the infected *Cucumis sativus* seedlings were placed to the laboratory greenhouse and the disease was expected to manifest. We maintained high humidity and temperature. On the 9-10th day, the affected plants were counted [16]. The degree of damage to plants was assessed on a 5-point scale. Derived the average damage score by existing methods [17].

The prevalence of the disease was taken into account by the number of sick leaves or its individual organs (leaves) in relation to all recorded leaves, expressed as a percentage. The intensity, or degree, of plant damage was determined by the area of the affected surface of leaves or by the intensity of manifestation of disease symptoms (visually) (Fig. 1).

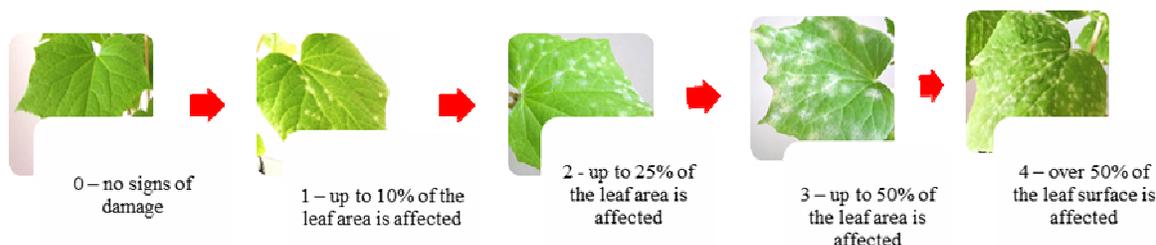


Fig. 1. Intensity of disease development on leaves (powdery mildew score)

The combined percentage-point scale (CIMMYT) was used to assess the manifestation of powdery mildew. Such a scale is compiled in accordance with the following groups of the intensity of the lesion in points: 1 - 2 - depression of the disease; 3 - moderate development; 4 - epiphytotic. The form for recording the results of accounting is a table. The degree of development, the prevalence of the disease and the biological effectiveness of protective measures were calculated using standard formulas (1-4).

The degree of development of the disease

$R = \sum (a \times b) \div N$, where R is the development of the disease, % or points; $\sum (a \times b)$ - the sum of the products of the number of diseased plants (a) by the corresponding percentage or damage score (b); N is the total number of plants in the sample, pcs.

(1)

Prevalence is the number of diseased plants as a percentage of the total surveyed in the area of the site. The prevalence (P, %) was calculated after counting sick and healthy plants in the sample according to the formula

$P = 100n / N$, where n is the number of diseased plants in the sample;

(2)

N is the total number of examined plants in the sample.

When taking into account the degree of damage to plants by point the scale is converted from a point grade to a percentage according to the formula into which the indicator k is entered - the highest score of the accounting scale. This formula has the following form: $Pa = ab \cdot 100 / Hk$

(3)

other designations of indicators are the same as in the previous formula.

The biological effectiveness of protective measures was assessed by comparing the infestation of plants on treated and control plants. The difference in the infestation of control and treated plants (BE, %) is determined by the formula

$BE = (100 (Bk - B0)) / Bk$, where

(4)

Bk is an indicator of the prevalence or development of the disease in the control plot;

B0 - a similar indicator on the experimental site.

Mathematical processing of the obtained data was carried out according to the method of one-way analysis of variance.

RESULTS AND DISCUSSIONS

The causative agents of powdery mildew based on the conidial stage have been identified and a suspension has been created for artificial infection

It was found that *C. sativus* powdery mildew in greenhouses of the Republic of Moldova is caused by two species of marsupial fungi - *Erysiphe cichoracearum* DC (*Golovinomyces cichoracearum*) and *Sphaerotheca fuliginea* Poll (*Podosphaera xanthii*). Powdery mildew pathogens in the greenhouse can be stored in the form of clestothecia (*P. xanthii*) and conidia (*G. cichoracearum*). However, in 2020, *C. sativus* disease in the greenhouse was caused only by the fungus *G. cichoracearum*, which we examined microscopically and recorded in the photo (Fig. 2).

It is known that conidia of pathogens on a dead substrate remain viable for different periods of time. Thus, *P. xanthii* conidia lose their ability to germinate within 7 days, and *G. cichoracearum* germinated 2 months after leaf break. Infection of healthy cucumber plants was successful with *P. xanthii* conidia 5 days after leaf break; conidia *G. cichoracearum* - after 35. The incubation period for both species at a temperature of 20-22°C and a relative humidity of 70-85% was 6-8 days. The strong spread of the disease is facilitated by sharp fluctuations in temperature and humidity, watering with cold water. The harmfulness of powdery mildew increases in dry and hot weather, when the turgor of the plant decreases and the penetration of the pathogen into the plant through the integumentary tissues becomes easier. In 2020, only conidia of *G. cichoracearum* we detected. In the middle of summer (July, August), due to the formation of high temperatures (40-60°C) in the greenhouse, the plants were completely sterilized and the powdery mildew disappeared.



Fig. 2. The identification of *Cucumis sativus* powdery mildew pathogens based on the conidial stage: a, b) a leaf of *Cucumis sativus* affected by powdery mildew; c) conidia of cucumber powdery mildew disease (*Golovinomyces cichoracearum* (de Candolle) Heluta)

This phenomenon is confirmed and explained by the work of researchers from the Czech Republic, who determined the effect of heat shock treatment of plants (40,5°C, 2 hours) on the development of powdery mildew on *Solanum* spp. In the treated tomato plant, there is a suppression of the development of pathogenic microorganisms, an increase in the concentration of jasmonic acid (JA), abscisic acid (ABA) and peroxidase activity (POX). The development of biochemical reactions to infection was enhanced [18]. In September, after the daytime temperatures dropped, infestation in our greenhouse appeared in large numbers on *Cucumis sativus*. Conidia were collected and experiments with artificial infection of seedlings were continued.

Conidia collected from diseased cucumber plants in a greenhouse were transferred to glass slides, photographed and used to create a suspension. Conidia were examined microscopically. Powdery mildew pathogens on *C. sativus* were identified according to Sokolov's methods. Fresh conidia of *G. cichoracearum* are cylindrical in shape. Conidia are arranged on conidiophores in chains. The spores of the fungus were washed off with distilled water, and the solution was filtered. The concentration of the suspension was 100 thousand conidia in 1 ml of solution. The plants treated with the extracts were infected with powdery mildew pathogen after 72 and 4 hours.

Determination of the fungicidal and immunomodulatory effect of treatment with plant extracts

As a result of the research, it was noted that after the treatment of diseased *C. sativus* seedlings with plant extracts, the spread of the disease through the plant stopped. At the same time, after infection, the control plants were covered with colonies of powdery mildew, and the re-infection of new leaves led to the death of *C. sativus* plants within a month. It was found that the biological effectiveness of the rhubarb root and leaf extracts composition (V5 and V6) when treated 4 hours before infection of seedlings was maximum (86,7%, 100%) and the plants were completely protected (Fig. 3).

With an interval between infection and treatment of 72 hours, the biological effectiveness of variants Variant 5 and Variant 6 also remained high (80%, 86,7%). The results of the experiment confirm that these compositions not only have a local, antiseptic effect on the plant pathogens of powdery mildew, but also activate immunity and development in the leaves. In other words, we proved the presence of fungicidal and immunostimulating activity of the composition of rhubarb root and leaf extracts.

In a laboratory greenhouse, when artificial infection was used on cucumber seedlings, stable fungicidal and immunostimulating activity of rhubarb root extract (Variant 1 and Variant 2) was established, which reduced the degree of damage to

cucumber seedlings by an average of 80% compared to control. It has been proven that the biological effectiveness of rhubarb root extract (from 73,3% to 86,7%) does not depend on the time between treatment and infection, but increases in direct proportion to the concentration. This indicates that the extract has immunostimulating (stimulating the immune response to infection with powdery mildew) and fungicidal (destruction of phytopathogen conidia, directly, on the infected leaf) types of activity (Table 1).



a)



b)

Fig. 3. The degree of plants damage by powdery mildew: a) control, untreated plants; b) plants treated with a combination of extracts 1% R + 0,5% L (Variant 6)

The extracts, and their compositions, had a stimulating effect on plant growth and the number of flowers, while the number of leaves remained within the margin of error with the control variant. Thus, it was found that the extract from rhubarb leaves (Variant 4 – 0,5% L) had a stimulating effect on plant growth (34,9% more than the control values), without affecting the number of leaves and flowers. Plant height (42,3 cm and 44,7 cm) and the number of flowers (8,3 pcs. and 11,5 pcs.) Indicate the presence of phytostimulating activity of compositions Variant 5 and V6 (Figure 4.)

Based on the analysis of the results, we concluded that the compositions are multifunctional. Thus, the bioactive substances of the *Rheum officinale* leaf extract (organic acids and quercetin) in combination with the main active substances of the rhubarb root extract (emodin and quercetin) had an active fungicidal, immunostimulating and phytostimulating effect on *Cucumis sativus* seedlings. The compositions demonstrated, in addition to direct fungicidal action, also high efficiency in stimulating the resistance of crops to the pathogens *Golovinomyces cichoracearum* (de Candolle) Heluta, as well as in stimulating the physiological parameters of *Cucumis sativus* seedlings. The use of polyfunctional compositions based on extracts of *Rheum officinale* is a promising line of research for the development of plant protection products and is cost-effective, since the extracts are created from the wastes of rhubarb stalks. Eco-friendly means of protection reduce the number of chemical treatments on the cucumber crop. The creation and use of remedies based on extracts will reduce the pollution of the ecosystem, and stimulating immunity will allow plants to reduce energy costs for protecting against pathogens and save energy for growth, development and formation of fruits. The combination of single- and multicomponent pesticides in a mixture or in rotation can provide additive or even synergistic interactions, while achieving a high level of disease control with a reduced dosage of each individual fungicide, which in turn reduces the risk of developing pesticide resistance among plant pathogenic strains. Extracts represent a worthy alternative for synthetic plant growth regulators due to their environmental friendliness, ease of use, effectiveness and safety.

Table 1. Determination of the fungicidal and immunomodulatory effect of treatment with plant extracts in the control of *Cucumis sativus* L. seedlings powdery mildew

Variant	powdery mildew score	the intensity of the defeat, %	biological effectiveness, %
Interval 4 hours			
Control	1,5	30	
Standard	0,6	12	60
V 1 0,5% R	0,3	6	80
V 2 1% R	0,2	4	86,7
V 3 0,1% L	0,4	8	69,2
V 4 0,5% L	0,4	8	69,2
V 5 0,5% R+0,1% L	0	0	100
V 6 1% R+0,5% L	0,2	4	86,7
V 7 0,5% N	0	0	100
V 8 0,5% R+0,1% L+0,5% N	0	0	100
V 9 1% R+0,5% L+1% N	0	0	100
HCP _{0.05}		4,4	
Interval 72 hours			
Control	1,5	30	
Standard	0,6	12	60
V 1 0,5% R	0,4	8	73,3
V 2 1% R	0,2	4	86,7
V 3 0,1% L	1,4	28	6,7
V 4 0,5% L	1,5	30	0
V 5 0,5% R+0,1% L	0,3	6	80
V 6 1% R+0,5% L	0,2	4	86,7
V 7 0,5% N	0,1	2	93,3
V 8 0,5% R+0,1% L+0,5% N	0	0	100
V 9 1% R+0,5% L+1% N	0	0	100
HCP _{0.05}		7,9	

Legend. Extracts: R - *Rheum officinale* root; L - *Rheum officinale* leaves; N - *Azadirachta indica* A. Juss seeds

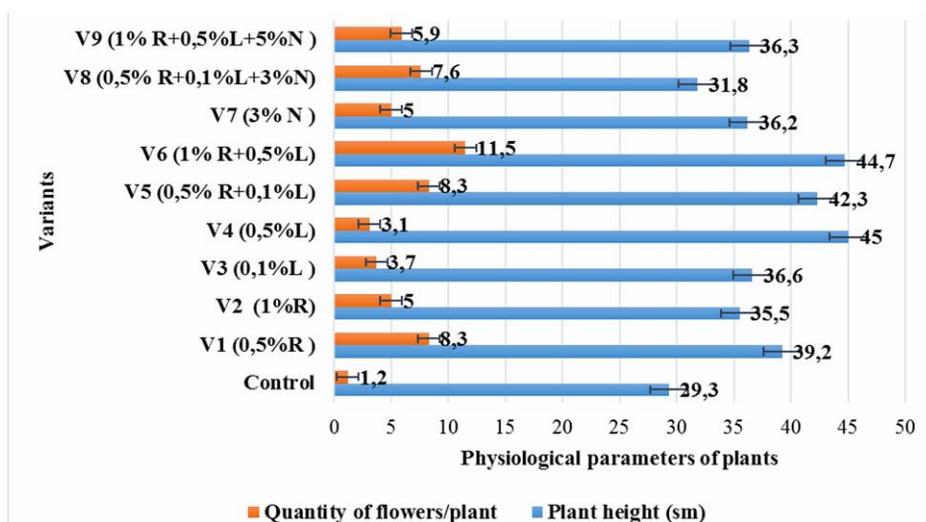


Fig. 4. Determination of the phytostimulating effect of treatment with plant extracts and their compositions on the physiological parameters of *Cucumis sativus* L seedlings
Legend. Extracts: R - *Rheum officinale* root; L - *Rheum officinale* leaves; N - *Azadirachta indica* A. Juss seeds

CONCLUSIONS

1. A method has been developed for obtaining an expanded spectrum of bioactive substances using extraction (70% ethanol) of shredded rhubarb roots and leaves in a water bath, followed by maceration;

2. The pathogens of powdery mildew on cucumbers *Golovinomyces cichoracearum* (de Candolle) Heluta, class *Ascomycetes*, the kingdom of *Mycota* based on the conidial stage were identified, examined microscopically and fixed in the photo, and a suspension for artificial infection was created;

3. We found that *Rheum officinale* root and leaf extracts showed three types of activity - immunostimulating, fungicidal and phytostimulating. It has been proven that the biological effectiveness of the extract from rhubarb root (73,3-86,7%) and neem seeds (93,3-100%) does not depend on the time between treatment and infection, but increases in direct proportion to the concentration. The biological effectiveness of the neem extract (100%) and the composition of the extracts of rhubarb root and leaves (86,7-100%) when treated 4 hours before the infection of seedlings was maximum. Rhubarb leaf extract is antiseptic and has no immunostimulating properties.

4. We have proven that *Rheum officinale* plant extracts (leaves and roots) had an active phytostimulating effect on cucumber plants. The composition 1% R + 0,5% L showed the maximum phytostimulating effect, which significantly increased plant growth by 34,5% and increased the number of flowers by 89,6% in comparison with the control variant.

ABSTRACT

In order to produce organic products, we conducted research on the properties of herbal extracts based on *Rheum officinale* Baill. (*Polygonaceae*) and *Azadirachta indica* Juss. (*Meleaceae*). The purpose of the work was to determine the possibility of using bioactive substances of plant extracts as a means of protecting the seedlings of *Cucumis sativus* L. from powdery mildew. We studied microscopically, powdery mildew pathogens *Golovinomyces cichoracearum* (de Candolle) Heluta, (*Ascomycetes*) identified on cucumbers and created a suspension for artificial infection. Extracts of *R. officinale* roots and leaves exhibited immunostimulating, fungicidal and phytostimulating types of activity. We found that the biological effectiveness of rhubarb root extract (73,3-86,7%) and neem extract (93,3-100%) does not depend on the time between treatment and infection, but increases in direct proportion to the concentration. It was found that, under greenhouse conditions, the biological effectiveness of the neem extract (100%) and the composition of rhubarb root and leaf extracts (86,7-100%) when treated 4 hours

before the seedlings were infected, was maximum. Rhubarb leaf extract is antiseptic and has no immunostimulating properties. Bioactive substances of plant extracts had an active phytostimulating effect on plants of *Cucumis sativus* seedlings. The composition V6 (1% R + 0,5% L) showed the maximum phytostimulating effect, which significantly increased plant growth by 34,5% and increased the number of flowers by 89,6% in comparison with the control variant. Extracts represent a worthy alternative for synthetic plant growth regulators due to their environmental friendliness, ease of use, effectiveness and safety.

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